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Improving CNS Delivery to Brain Metastases by Blood-Tumor Barrier Disruption

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Abstract

Brain metastases encompass nearly 80% of all intracranial tumors. A late stage diagnosis confers a poor prognosis, with patients typically surviving less than two years. Poor survival can be equated to limited effective treatment modalities. One reason for the failure rates is the presence of the blood-brain (BBB) and blood-tumor barriers (BTB) that limit the access of potentially effective chemotherapeutics to metastatic lesions. Strategies to overcome these barriers include new small molecule entities capable of crossing into the brain parenchyma, novel formulations of existing chemotherapies, and disruptive techniques. Herein, we review BBB physiology and BTB pathophysiology. Additionally, we review the limitations of routinely practiced therapies and three current methods being explored for blood-brain/blood-tumor barrier disruption for improved delivery of chemotherapy to brain tumors.

Keywords

Blood-brain barrier; Blood-tumor barrier; permeability; disruption

Brain Metastases and Treatment Failure

Brain metastasis is an overwhelming morbidity of late stage cancer progression. Central nervous system (CNS) metastases occur in approximately 10% of all cancer types [1]. Recent increases in brain metastases are thought to be caused by improved control of systemic disease and increasingly sensitive imaging modalities [2]. Patients with CNS disease typically succumb within two years of diagnosis [3-5]. Therapies for brain lesions are mostly palliative, and rarely ever curative. These therapies include bulk surgical resection of the tumor(s), radiation therapy (either whole-brain and/or stereotactic), and/or systemic chemotherapy [6]. The blood-brain barrier (BBB), the brain's innate defense system against blood delivered harmful substances, prevents delivery of most all efficacious systemic chemotherapies into brain tissue [7]. To improve efficacy of chemotherapeutics and

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small molecules, a way to bypass the BBB is necessary. In this article we discuss the utility and potential mechanisms of three widely explored BBB disruption techniques aimed at improving chemotherapeutic distribution to brain lesions. Understanding these techniques may result in the ability to progress treatment and affect survival of patients with this grim diagnosis.

The Blood-brain Barrier

The BBB's unique properties occur as a result of specific interactions between endothelial cells, pericytes, astrocytes, microglia and neurons, and their molecular components as seen in Figs. 1, Key Fig. 3a [8]. Proper regulation and function of the BBB is dependent on uninhibited interaction and communication between these cells.

Endothelial Cells

Brain microvascular endothelial cells (EC) form the foundation layer of the BBB and are crucial to the maintenance of its integrity. The ECs of the BBB are polarized in structure, as their luminal and abluminal surfaces have diverse biochemical and functional features; e.g. increased luminal γ-glutamyl transpeptidase expression [9]. The specialized BBB ECs have a high degree of expression of various transporters, including P-glycoprotein (P-gp, ABCB1), breast cancer resistance protein (BCRP, ABCG2), multi drug resistance protein, and various nutrient transporters [9]. These transporters move nutrients into the brain and efflux waste and other molecules out of the brain. Efflux pump expression is a major obstacle in overcoming drug delivery to the brain.

One of the most crucial features of ECs is their expression of tight junctions (TJ), which stabilizes the integrity of the BBB. The expression of TJ's is induced by pericytes and results in a non-fenestrated vasculature preventing any unwanted "leaking" of luminal contents into the parenchyma of the brain. The TJ proteins are comprised of various transmembrane proteins including claudins, occludins, junctional adhesion molecules and accessory proteins, such ZO-1 and ZO-2 [10]. Another important trafficking molecule in normal BBB anatomy is major facilitator superfamily domain 2a (Mfsd2a). This protein is important for development of a functional BBB and is required for movement of docosahexaenoic acid into brain tissues [11, 12]. Importance of this transporter in BBB integrity and functionality is demonstrated by mice with genetically removed Mfsd2a that have decreased docosahexaenoic acid transport and increased disruption of the vascular barrier in brain [13].

Pericytes

Pericytes share the basement membrane with ECs and attach to them by 'peg-socket' junctions within the cerebral vasculature [14, 15]. During developmental stages and adult life, pericytes are recruited to EC of the BBB through several signaling methods, primarily the platelet derived growth factor-β pathway [14].

The presence of pericytes is critical for proper BBB function and development. These accessory cells directly influence permeability of the BBB by inducing EC TJ formation [16]. Next, pericytes regulate cerebral blood flow and waste clearance, disruption of which is

associated with multiple brain pathologies, such as Alzheimer's [15, 16]. Pericytes are shown to polarize astrocytic end-foot processes surrounding the BBB, and further are shown to regulate EC gene expression, increasing their viability through the Bcl2l2 pathway [17, 18].

Astrocytes

Astrocytic end-feet processes surround the BBB almost entirely. Their end-feet connect to the basement membrane through junctional molecules, including dystroglycan as well as channels like aquaporin 4, a molecule shown to maintain water homeostasis in the brain [10, 19]. Astrocytes play several roles in the regulation of the BBB. They assist in regulation of cerebral blood flow through Ca^{2+} signaling following neuronal perturbation [20]. Further, astrocytes are responsible for maintenance and formation of EC TJ. Sonic hedgehog, ang-1, and transforming growth factor signaling pathways influence this maintenance [10, 21]. Lastly, astrocytes directly impact vascular growth and proliferation through ang-1 and vascular endothelial growth factor (VEGF) secretion [10, 22, 23].

Microglia

Microglia are the resident immune cells of the brain. These cells play a role in both pro- and anti-inflammatory responses. Depending on their pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes, they control inflammation through release of various molecular cytokines. Microglia are involved in angiogenesis, especially near EC tip cells, suggesting their influence in cerebral vascular development [24, 25]. However their role in maintenance of the integrity of a healthy BBB is still unknown.

The Blood-tumor Barrier

Of the primary cancers that migrate to the brain, lung, breast, melanoma, and renal cancers comprise the majority of metastatic brain tumors affecting ~50%, ~15%, ~10%, and ~5% of patients respectively [2]. Brain metastasis occurs when a circulating tumor cell, of a primary systemic tumor (i.e., breast, lung, melanoma, renal), detaches from the initial tumor mass and arrests in the brain microvascular capillary network, extravasates through the vessel wall into the perivascular space, and survives and proliferates into a new lesion [26, 27]. From initial metastatic colonization, the newly "seeded" brain metastatic tumor cells co-opt the brain vasculature eliciting neo-angiogenesis and microenvironment remodeling to promote tumor growth and further invasion. The newly formed neurovascular-tumor unit is termed the blood-tumor barrier (BTB, Fig. 2) and has differential properties concerning therapy pharmacokinetics and action in comparison to the intact BBB.

The BTB is inherently "leaky", lacking tight junctions and astrocytic-endothelial contacts resulting in significant heterogeneous permeability from lesion to lesion within the brain [28, 29]. As lesions continue to outgrow their oxygen supply, angiogenesis occurs driven largely by VEGF. These new vessels are inherently leaky compared to the BBB phenotype. Dynamic angiogenesis during metastatic progression is different among brain lesions, which is thought to contribute to the heterogeneity in tumor permeability to chemotherapy. Additional contributions to increased permeability of the BTB include the lack of

physiological TJ protein expression causing fenestra and discontinuous endothelia [28, 30]. Inconsistencies of junctional protein expression can allow for the passive permeability of cytotoxic therapies into tumor tissue. Interestingly mfsd2a is down-regulated at the BTB and promotes brain metastatic outgrowth due to lack of astrocytes promoting endothelial expression of mfsd2a, further contributing to BBB leakage in brain tumors [31].

Efflux mediated by P-gp (ABCB1) and BCRP (ABCG2) at the BBB limits distribution to normal brain of most chemotherapeutic agents. In the BTB setting, P-gp and BCRP have been found to be increased at the luminal membrane, as well as in the plasma membrane of tumor cells [32-34]. In preclinical mouse models, Elmquist and colleagues have demonstrated the active efflux of a host of agents used to treat melanoma and lung cancer brain metastases [32, 35, 36].

Other cellular and molecular properties of the BTB are prompted by astrocytes, pericytes and microglia. Astrocytes function to support and protect neuronal cells from damage and apoptosis through secretion of inflammatory cytokines such as TNFα, IL1, and IL6. Release of these cytokines encourages tumor proliferation and survival.[37] Additionally, astrocytes release exosomes containing miRNA-19a, which serves to induce loss of PTEN and promote further outgrowth and invasion of tumor cells within the brain [37, 38]. Microglia in the brain tumor microenvironment are known to secrete multiple growth factors and cytokines, such as TGFβ, TNFα, IL1, IL6, VEGF, EGF, and many metalloproteinases [39]. The molecular entities secreted by microglia promote tumor proliferation and invasion, as well as support angiogenesis [39]. Microglia cell populations also support colonization through the Wnt pathway, an effect attenuated with addition of Wnt inhibitors [40]. Pericyte subpopulations are known to contribute to BBB integrity and therefore permeability. Desmin + pericytes are found in high concentrations in brain metastases and their presence is associated with high permeability [41].

Taken together, the distinct physical and molecular impedance the BTB plays in cancer treatment may seem insurmountable. In fact, the BTB, even in the presence of heterogeneous disruption, limits drug accumulation to the degree that there is limited apoptosis and cytotoxicity in nearly 90% of metastatic lesions in experiments utilizing preclinical models of breast cancer brain metastasis [42-47]. Inability of drugs to distribute to brain tumor tissues has led to the progression of techniques aimed at disrupting the BBB.

BBB/BTB Disruption for Increased Therapeutic Potential.

Disruptive CNS barrier techniques have increasingly become a research focus. Three highly investigated areas include the use of focused transcranial ultrasound (t-FUS) coupled with intravenously delivered microbubbles, hyperosmotic agents, and to a lesser degree radiation therapy that elicits transient changes in BBB permeability. Each of these applied to the treatment of metastatic brain lesions may lead to increased drug distribution and improve efficacy of many approved therapeutics. A list of ongoing or completed clinical trials utilizing disruption techniques can be found in Table 1.

Focused Transcranial Ultrasound

Transient focused transcranial ultrasound (t-FUS) with concurrent administered intravenous microbubbles has been investigated as it can increase barrier permeability and improve distribution of CNS targeted therapeutics. Preliminary studies on mechanisms of BBB disruption indicate that the minimally invasive low intensity t-FUS coupled with the acoustic cavitation produced by the microbubbles cause molecular changes in tight junctions through decreased expression of claudin-5, ZO-1 and occludin, which enable the paracellular transport of genomic and chemical therapeutics as well as initiate inflammatory responses associated with damage-associated molecular patterns (Fig. 3b) [48, 49]. Combined with the higher hydraulic conductivity of interstitial fluid to the solid tumors, these changes have been used not only for higher tumor targeted delivery of many small molecule therapeutics but also for genes and immune cells [50-52].

Ultrasound influences the rate and extent of microbubble cavitation through its physicochemical properties that may lead to the production of stable or inertial cavitations. Under the influence of the FUS, microbubbles can undergo harmonic or non-harmonic oscillations which are responsible for the transient tight junction disruption; or undergo expansion and eventual collapse which can result in supplemental leakage or permanent damage [53, 54]. The amplitude and frequency of the ultrasound govern the mechanical index of the microbubbles and lead to enhanced disruption by specialized mechanisms including the push-pull action mediated broadening of ECs, high shear stress through micro-stream production, acoustic radiation, and pressure gradient mediated microbubble displacement [53, 55]. However, when microbubbles undergo unstable expansions and collapse it can lead to high EC lining pressure which may cause fragmentation of microbubbles resulting in mircro-jets and shock-waves. Additionally, microbubbles may also undergo free radical formation depending on microbubble lipid content and the degree of cell membrane permeabilization [56]. Altering the parameters of microbubbles enables their use as drug delivery devices as shown by a recent study that used a novel nitrogen based folate conjugated microbubble system encapsulated with methotrexate to increase its site-specific delivery and thus drug efficacy using high intensity focused ultrasound [57].

A recent study investigated the BBB/BTB penetration and cellular uptake of small (Doxorubicin) and large (ado-trastuzumab emtansine) molecules for an orthotopic brain metastasis of HER2 positive breast cancer model [50]. The study demonstrated that the small hydrophobic molecule showed a much higher (7-fold) concentration in the extravascular compartment along with high tumor penetration when FUS was used as opposed to control. In contrast, despite showing a 2-fold increase in the extravasation and slightly higher tumor penetration, the long (4-6d) drug circulation and transient effect of ultrasound diminished the overall effect when compared to control on day 5. Another study investigated the antitumor efficacy of polymeric polysorbate 80 modified paclitaxel nanoparticles and found an increase in the median survival of U87-Luc glioma-bearing mice to 37 days when to the control’s 26 days [58]. They demonstrated that the ultrasound mediated reduction in P-gp expression and tight junction disruption as well as apolipoprotein mediated endocytosis was responsible for the enhanced permeation of the nanoparticles. These pre-clinical studies in animal models have shown high efficacy leading

to multiple trials to test the use of ultrasound in drug delivery for neurological diseases including Alzheimer's [i], Parkinson's Disease [ii] with dementia and multiple gliomas [iii].

Despite promising results, there are challenges such as high inertial cavitations of the microbubbles that cause vascular and tissue damage, reliance on expensive techniques like contrast magnetic resonance imaging to detect disruption, and lack of normalized experimental conditions. A study to reduce the inertial cavitation and provide an alternate treatment modality used closed loop cavitation mechanism to accurately provide 274.3 kHz of ultrasound; increased both survival and tumor regression by increased doxorubicin delivery in glioma bearing rats [59]. An alternate semiautomatic approach to deliver the ultrasound used unfocused ultrasound devices implanted in patients with glioblastoma. The study correlated local acoustic brain pressures with signal enhancement of greater than 10 percent observed through ultrasound which was more in gray matter [iv].

Radiation Therapy

The effects of radiation therapy on the BBB have been studied since the early 1980s [60]. However, the precedent of radiation therapy with subsequently timed chemotherapy was first suggested in 2002 by van Vulpen et. al [61]. The dose dependent response and time course of disruption of the BBB following radiation therapy is highly debated with the existence of contradictory reports. The pathophysiological changes following BBB disruption induced by radiation have been segregated into two main categories, acute and late phases [62-64]. Acute effects are thought to occur within the first 24 hours following cranial irradiation and, late effects are those described thereafter [65].

Mechanisms of radiation induced permeability (Fig. 3c) during the early stages after therapy include EC death and an increase in neuro-inflammation. Microvascular cell density and tight junction protein, ZO-1, expression was shown to decrease from 1 to 180 days following a single 10Gy whole brain radiotherapy dose [66]. A similar study reported EC density decreases at a single 10Gy dose are greatest at 10 days following radiation therapy [67]. Another study indicating the death of ECs as an early event following cranial radiation observed an increase in apoptotic ECs peaking at 12 hours after radiotherapy at doses ranging from 5Gy to 100Gy [68]. From these data, it appears evident that changes at the endothelial level occur, but the exact timing and mechanism are not clear.

The neuro-inflammatory response following radiation insult is characterized by activation of astrocytes, microglia, ECs, and their inflammatory mediators. Astrocytic and microglia activation following cranial exposure to radiation have been indicated as early as 4 hours and as late as 6 months following radiation treatments demonstrated by increased GFAP and CD11b staining [69, 70]. While these indicators of cellular activation are present, a number of cytokines and adhesion molecules are also variably increased following radiotherapy. In studies by Hong et al. and Kyrkanides et al. at four hours post radiation treatment, increases in CNS levels of TNFα, IL-1 β, and IL-6 were shown [71, 72]. In a similar study, Ruimeng

i. https://clinicaltrials.gov/ct2/show/NCT03671889
ii. https://ClinicalTrials.gov/show/NCT03616860
iii. https://ClinicalTrials.gov/show/NCT03608553
iv. https://ClinicalTrials.gov/show/NCT02253212

et al. demonstrated the capacity for radiation therapy, at a dose of 50Gy, to increase immune cell activation and a panel of cytokines, including TNF-α and IL-6, at 12-weeks post treatment [73]. These research data suggest a critical role of the neuro-inflammatory response to radiation.

Taken together, the physiological responses to radiation alter the BBB/BTB in a manner which increases permeability. Data on the time course of increased permeability have been reported, but are variable among studies. Wilson et al. reported significantly altered permeability at 24 and 48 hours following cranial irradiation with a single dose of 20Gy, which could be rescued with anti TNF-α treatment [63]. Confirming this, a study of the rat BBB saw significant increases in permeability peaking at 24 hours posttherapy at a single dose of 20Gy to 4.4-, 10-, 38.2-, and 70-kDa FITC-dextran molecules [74]. Interestingly in Yuan et al.'s study, the time dependent increase in BBB permeability correlated well with an increase number of rolling leukocytes at the BBB, suggesting an increase in ICAM-1, a molecule expressed on the luminal surface of the BBB to aid in leukocyte trafficking to the brain parychema during an immune response [74]. Another study confirming early BBB disruption as soon as 24 hours following irradiation with single doses of 20 and 40Gy [75]. Each of these studies used a different means of irradiation, resulting in a specific dose rate for each respective study. This may provide information regarding the effect of dose rate on permeability related outcomes.

Another factor potentially contributing to permeability of the BBB/BTB may be fractionation schemes. Using daily doses of 4Gy for 3 consecutive days, Crowe et al. demonstrated enhanced permeability of irradiated tumors at 24 hours post-treatment compared to their contralateral sham treated counterparts when analyzed using DCE MRI [76]. Fractionation may elicit potentially altered permeability outcomes. Additionally, the particular mode of irradiation may play a role in pathophysiologic response to irradiation as well. When comparing broad beam radiation to micro-beam radiation therapy, Bouchet et all showed higher permeability increases in tumors treated with microbeam radiation therapy compared to those treated with conventional broadbeam radiotherapy at all time points, with a maximum at 7 days following radiation treatment [77]. Of note, there was increased permeability in lesions treated with BBRT compared to non-treated regions [77].

These studies all provide insight as to when the permeability changes may occur following radiation treatment. Contrary to this work another study by Murrell et al. noted that a dose of 20Gy in 2 fractions was not able to increase tumor permeability in a preclinical model of breast cancer brain metastasis [63]. Their work was subjected to only two time points however, one week and 11 days post radiation treatments. It is important to note that both authors may be suggesting the correct response. BBB/BTB opening following radiation therapy treatment may be transient or biphasic in nature, with points of high and low permeability in different phases, similar to that of stroke pathology [78].

Clinically there is evidence of breakdown of the BBB and BTB after radiotherapy as well. In a study of 30 patients receiving WBRT or SRS, with 64 analyzed metastatic lesions, radiotherapeutic treatments improved the permeability of initial low leaky tumors at 2 weeks and 1 month post therapy [79]. However, there was little or decreased permeability in

initially very leaky metastases [79]. Zeng and colleagues also showed that in NSCLC patients treated with WBRT and concurrent gefitinib therapy, increased drug penetration was observed in accordance with escalation of radiation dose [80]. Lim et al. saw increased gadolinium deposition in peri-tumoral areas in 44 glioblastoma patients, but no change in untreated areas, indicating BBB/BTB disruption following radiation therapy [81]. These date provide evidence for increased permeability following radiation, but none give information elucidating the time course or magnitude of increased permeability.

Hyperosmotic Agents

Pre-clinical and clinical strategies have targeted the transient loosening or disruption of the BBB to increase permeability of therapeutics by techniques such as ultrasound, radiation or hyperosmotic agents like mannitol. One of the earliest techniques to disrupt the BBB using hyperosmotic agents was described by Neuwelt et al; wherein hyperosmotic mannitol administered via an intra-carotid injection was used to reversibly disrupt the BBB in canines [82]. The work demonstrated that when methotrexate was administered after the hyperosmotic agent, the drug levels were significantly higher (nearly 5-9 times as compared to control) in the ipsilateral cerebral hemisphere and contralateral hemisphere [82].

Although subsequent studies have failed to identify a singular mechanism underlying the mannitol mediated disruption, multiple distinct phenomena have been proposed. The most widely accepted theory of BBB opening is dehydration of the ECs followed by vasodilation induced shrinkage or contraction of the cells due to altered intracellular calcium levels (Fig. 3d) [83]. The resulting tension along with the calcium dependent actin and cadherin interaction leads to the widening of the tight junctions by increased bulk flow and solute diffusion. Other factors like nitric oxide, inflammatory mediators, bradykinin and mannitol induced tyrosine phosphorylation of Axl and beta-catenin have been implicated to augment the BBB disruption; however the exact mechanism is still not understood [83, 84].

Despite facing early challenges like potential neurotoxicity, osmotic disruption has been successfully used in pre-clinical models for improving drug therapy. Pharmacological agents such as oligonucleotides that have poor brain delivery have improved distribution by hyperosmotic mannitol mediated BBB disruption [85]. The study further demonstrated a high dissemination of the oligonucleotide in the ipsilateral brain regions including the striatum, somatosensory cortex and thalamus upon co-administration of 25% mannitol and the oligonucleotide which was modified with a hydrophobic moiety. In addition, the striatum, thalamus, motor cortex, hippocampus and somatosensory cortex showed Huntington gene mRNA silencing even a week after the initial therapy administration.

Concluding Remarks

Disruption of the BBB/BTB by ultrasound, radiation or hyperosmotic agents appears to be a promising aid to the delivery of chemotherapy for brain metastases. Studies using these disruptive techniques have shown to have an auxiliary impact on the brain distribution of traditional therapy. However many questions still remain unanswered like the length and extent of its effect, translation to the clinic, cost to benefit and many more (see outstanding

questions). Still, these disruptive techniques in combination with chemotherapy offer a unique system to combat the otherwise poor prognosis of brain metastases.

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Glossary

Active Transport
The movement of molecules into the cell across the cellular membrane assisted by enzymes.

Blood-Brain Barrier
The physicochemical barrier existing at the interface between the systemic circulation (blood) and brain limiting the passive and active transport of small molecules, proteins, toxins, and other potentially pathogenic entities into the brain.

Blood-Brain Barrier Disruption
A physical opening, transient or persistent, of the BBB or BTB through a variety of mechanisms with the intent of increasing distribution of therapeutics into brain tissues.

Blood-Tumor Barrier
Similar to the BBB in healthy individuals, the BTB is the interface between the blood and metastatic or primary tumor cells. This barrier is inherently “leaky” due to lack of tight junctions and neo-angiogenesis induced by the tumor.

Brain Metastases
Tumors formed in the brain by cancer cells that have detached and migrated from a primary tumor site.

Central Nervous System
Comprised of the brain and spinal cord, this complex of nerves controls the activities of the body.

Endothelial Cells
Cells that line the interior (luminal) surfaces of blood and lymphatic vessels.

Focused Transcranial Ultrasound
The use of low frequency ultrasonic waves, penetrating through the cranium to target particular sites within the brain.

Glioblastoma
Also known as glioblastoma multiforme. A form of primary CNS tumor arising from one of the glial cell types.

Neo-angiogenesis
The growth of new blood vessels.

Passive Diffusion

The movement of molecules across a membrane or between cells without the need for energy. Molecules down a concentration gradient, from a high concentration to a lower concentration.

Radiation Therapy

The use of X-rays, or similar forms of radiation, in the treatment of cancer.

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Highlights

- The blood-brain and blood-tumor barriers are responsible for therapy failure in many patients having brain metastases.
- The blood-brain and blood-tumor barriers have specialized structures and efflux transporters which limit the brain permeation of majority of the drugs that are administered intravenously.
- The neurovascular unit has been implicated in certain inflammatory responses that can aggravate the tumor invasion; however their exact role has not been elucidated.
- The use of blood-brain and blood-tumor barrier disruptive mechanisms like radiation, ultrasound or osmotic agents; may transiently increase permeability which may improve therapeutic distribution to tumor.

To what extent can barrier efflux transporters be implicated in the failure of drug therapy?

Do different tumor types alter the blood-brain barrier integrity in discrete ways?

Are inflammatory mediators involved in propagating ultrasound and radiation mediated blood-brain barrier disruption?

Do barrier disruptive techniques have long term detrimental effects?

Can targeting the molecular structures in the blood-brain and or blood-tumor barrier reduce the effects of therapy failure due to tumor heterogeneity?

Can disruptive techniques like ultrasound and radiation affect tumor microenvironment and improve health outcomes?

Can inertial cavitations be completely eliminated in t-FUS to limit permanent damage?

How long does the blood-brain and or blood-tumor barrier remain functionally open after disruption?

Can the effects of osmotic disruption be controlled?

Will the effects of blood-brain and or blood-tumor barrier disruption be clinically relevant?

Can radiation and ultrasound be used to eradicate tumor masses directly without affecting healthy cells?

How long after blood-brain and or blood-tumor barrier disruption is the window of maximum permeability?

Can blood-brain and or blood-tumor barrier disruption with co-administration of drug therapy be made more cost effective?

Does the vascular and structural heterogeneity in the cranium attenuate effects of blood-brain and or blood-tumor barrier disruption?

How can blood-brain and or blood-tumor barrier disruptive techniques be modified for increased drug delivery to the other organs including the liver or kidney?

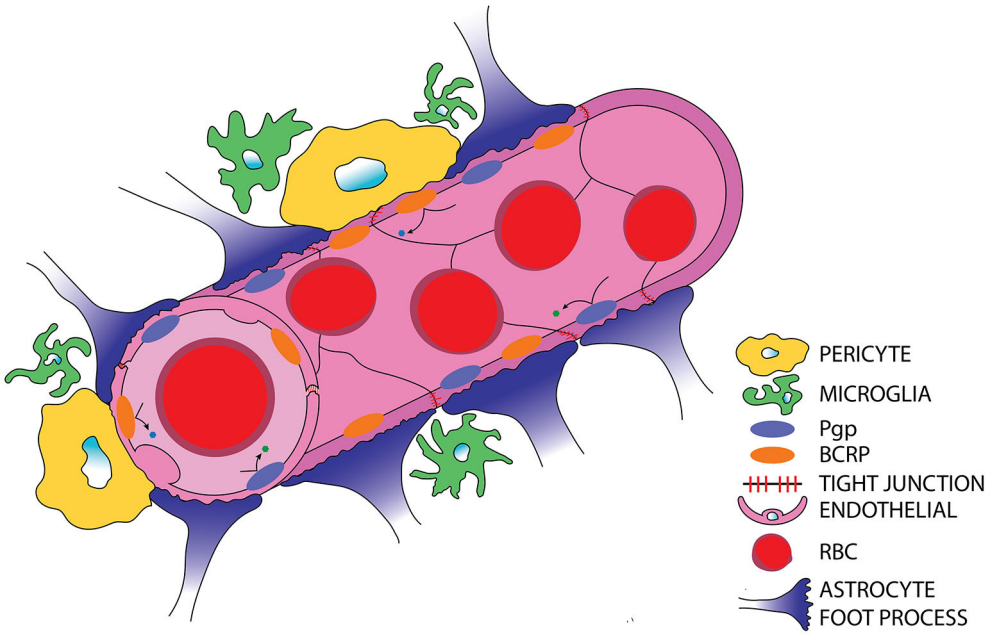

Figure 1. Normal blood-brain barrier anatomy and physiology.

Brain capillary endothelial cells are tightly held to one another through continuous tight junction proteins and express P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) efflux transporters. Astrocytic end-feet processes further seal and support BBB integrity. Pericytes further regulate cerebral blood flow and BBB permeability. Microglia, the brain's resident immune cells, can influence BBB permeability through inflammatory cascades and serve as the innate response to pathogens within the brain.

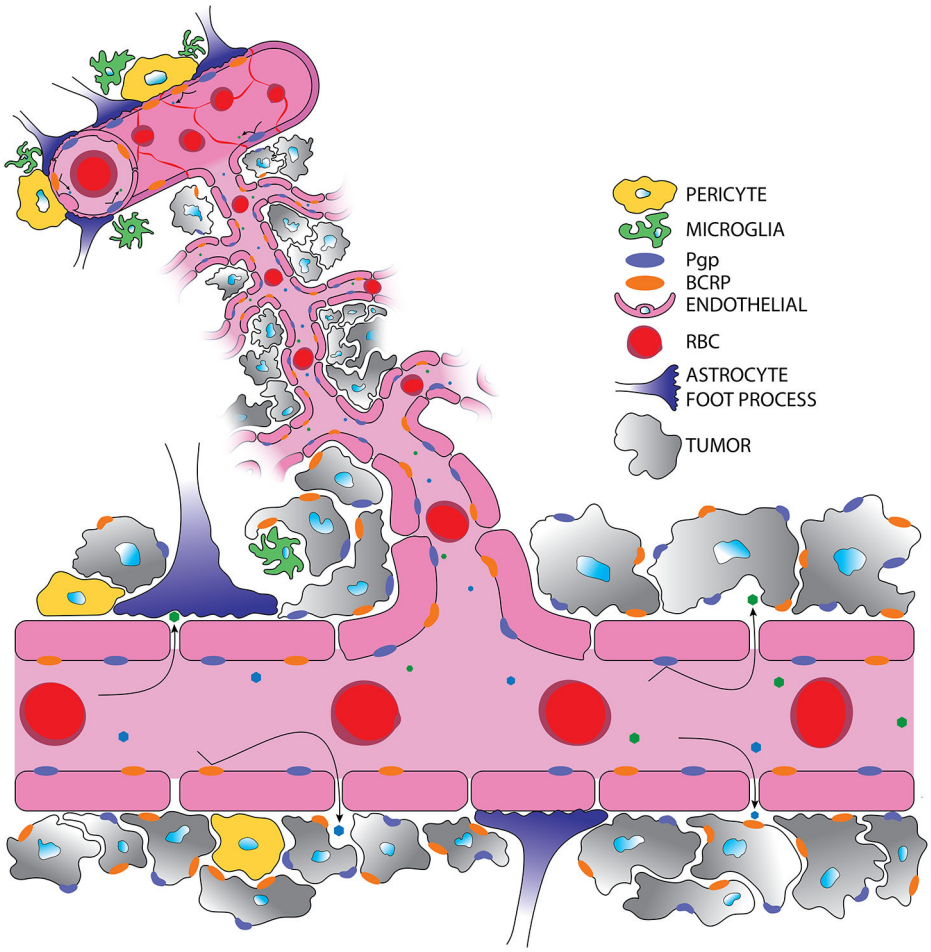

Figure 2. The blood-tumor barrier has altered anatomy and physiology.

Cancer cells coopt the cerebral vasculature and induce neo-angiogenesis resulting in fenestrated endothelia lacking tight junctional expression. Fenestrated, mal-formed vasculature allows for heterogeneous uptake of drug solutes. Cancer cells have increased expression of the P-gp and BCRP efflux transporters. At the BTB, less astrocytic end-foot processes and pericytes exist contributing to altered BTB integrity.

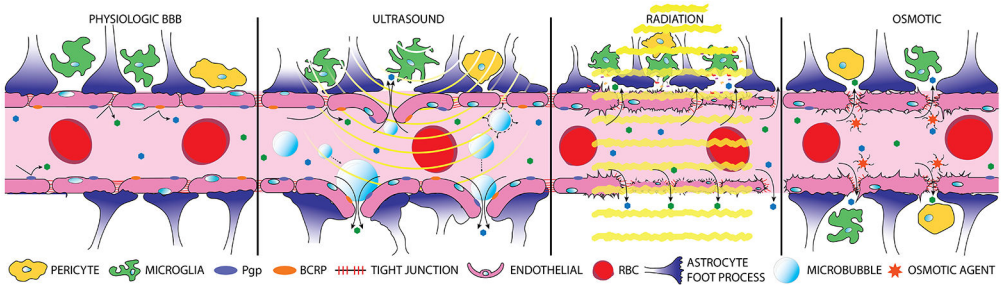

Figure 3, Key Figure. Blood-brain barrier disruption techniques.

Normal, undisrupted BBB with non-fenestrated endothelial cells sealed by tight junction proteins, further supported by astrocytic end-feet, pericytes, and microglia (a). Focused ultrasound (yellow curves) in combination with intravenously injected disrupts the BBB through cavitation and acoustic forces, ultimately leading to decreased molecular expression of tight junction proteins and an inflammatory response (b). Radiation therapy (yellow lines) disrupts the BBB through mechanisms of endothelial cell death and a neuro-inflammatory response from astrocytes and microglial cells (c). Hyperosmotic solutions are able to induce contraction and shrinkage of endothelial cells through a calcium dependent mechanism prompting widening of tight junctions (d).

Table 1.

BBB/BTB disruption techniques in ongoing or completed clinical trials.

Title	Trial number	Mode of disruption	Type of disease
Super-selective Intra-arterial Repeated Infusion of Cetuximab for the Treatment of Newly Diagnosed Glioblastoma	[v]	Intra-arterial Mannitol	Glioblastoma Brain Neoplasm, Malignant EGFR Gene Overexpression GBM
Blood Brain Barrier Disruption (BBBD) Using MRgFUS in the Treatment of Her2-positive Breast Cancer Brain Metastases (BBBD)	[vi]	ExAblate Model 4000 Type-2	Her-2 positive Breast Cancer, Brain Metastases
ExAblate Blood Brain Barrier Disruption (BBBD) for Planned Surgery in Glioblastoma	[vii]	ExAblate 4000 - Type 2	GBM
Assessment of Safety and Feasibility of ExAblate Blood-Brain Barrier (BBB) Disruption for Treatment of Glioma	[ii]	ExAblate Neuro Model 4000 Type 2.0	Glioblastoma
Blood-Brain Barrier Disruption Using Transcranial MRI-Guided Focused Ultrasound	[viii]	Transcranial ExABlate	Brain Tumor
ExAblate Blood-Brain Barrier Disruption for Glioblastoma in Patients Undergoing Standard Chemotherapy	[ix]	ExAblate 4000 type 2.0	Glioblastoma Multiforme
Safety of BBB Disruption Using NaviFUS System in Recurrent Glioblastoma Multiforme (GBM) Patients	[x]	Neuronavigation-guided focus ultrasound system (NaviFUS)	GBM, Neoplasm, glioma
Safety of BBB Opening With the SonoCloud (SONOCLOUD)	[iv]	SonoCloud	Glioblastoma, Glioma, Brain Tumor
MRI Study of Changes in Blood-Brain/Tumor-Barrier Permeability in Patients With Brain Metastases During and After Radiotherapy	[xi]	SRS, Fractinated WBRT, Fractionated SRS	Brain Metastases (Breast, Lung, Melanoma, etc.)

v. https://ClinicalTrials.gov/show/NCT02861898
vi. https://ClinicalTrials.gov/show/NCT03714243
vii. https://ClinicalTrials.gov/show/NCT03322813
viii. https://ClinicalTrials.gov/show/NCT02343991
ix. https://ClinicalTrials.gov/show/NCT03712293
x. https://ClinicalTrials.gov/show/NCT03626896
xi. https://ClinicalTrials.gov/show/NCT02031237